

Evaluation and Development of Low-Phytate Crops

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Phytic acid (Fig. 8-1 bottom) is the most abundant form of phosphorus (P) in plant seeds, accounting for 60 to 80% of seed total P (Lott et al., 2000). Phytic acid has a strong negative charge that is maintained over a wide pH range. Its six phosphate groups form "phytate" or "phytin" salts of various mineral cations, such as K^+ , Ca^{2+} , Mg^{2+} , Fe^{2+} , and Zn^{2+} (Tsao et al., 1997). Cereal grains phytates are primarily K/Mg salts, and contain >50% of the seed's total K and Mg. These cations, along with phytate's P and *myo*-inositol, form a pool used by seedlings during the early stages of development.

Phytate breakdown occurs in germinating seeds. In addition to the release of cations, phytic acid is hydrolyzed into inorganic P and *myo*-inositol by a class of nonspecific phosphomonoesterases called phytases (Reddy et al., 1989). Phytases catalyze the stepwise removal of the inorganic orthophosphate from phytic acid. There is a small amount of enzymatic activity in nongerminated seeds, but phytase-encoding genes are highly expressed during germination.

Much of the interest in phytic acid has been based on its role in human and animal nutrition. The human digestive tract contains little endogenous phytase activity. Some phytate complexes are insoluble, whereas others are soluble. The soluble phytic acid forms new complexes with minerals that are present in the gut (Schlemmer et al., 1995). This process decreases the bioavailability of P and cations, especially iron and zinc, stored as a part of the complex. In the case of iron, the negative impact of dietary phytic acid is on iron consumed concurrently in a meal. However, the impact of dietary phytic acid on zinc differs from iron in that phytic acid in the gut binds to both the zinc consumed concurrently in a meal and to endogenous zinc that is secreted into the gut along with digestive enzymes. Humans consuming plant-based diets rich in phytate are at risk for iron and zinc deficiency (Mendoza, 2002). More than half the population of the developing world receives inadequate micronutrient levels in their diets (Bouis, 2003). Two of the most widespread micronutrient deficiencies are that of Fe and Zn. Iron deficiency affects 3700 million people (Welch, 2002), and an estimated 49% of the human population is at risk for inadequate zinc in their diet (World Health Association data discussed in Brown et al., 2001).

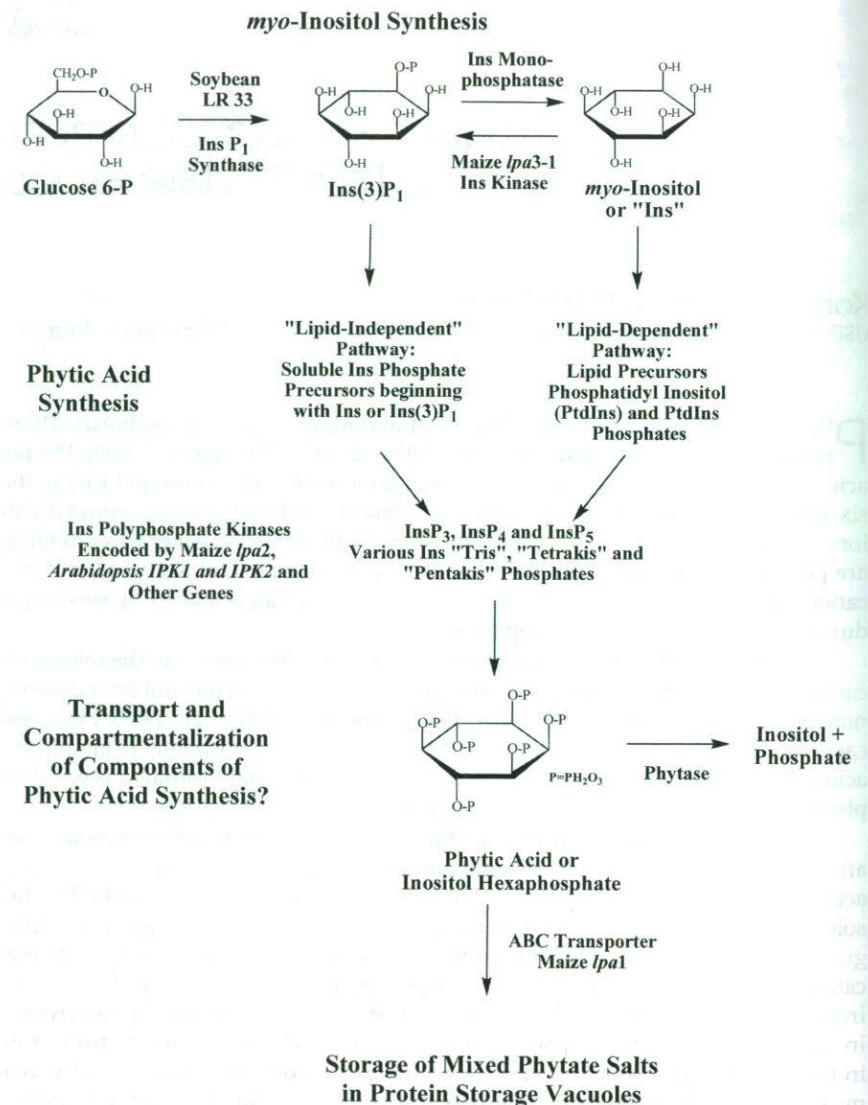


Fig. 8-1. Steps in the phytic acid synthesis pathway and transport and storage of phytate.

In mature cereal grains, phytates are localized to the germ and aleurone layer, outer layers of the grain that are removed during milling of rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.). Since phytates contain most of the grain's P, K, and Mg, and smaller amounts of other nutritionally important minerals, these valuable nutrients are removed during the production of white rice and refined wheat flour. This represents a second way cereal phytates may affect the nutritional quality of staple foods; white rice and products made from refined white wheat flours have low levels of P and minerals.

Seed-derived phytic acid may also have health-beneficial roles as an antioxidant and anticancer agent (Graf et al., 1987; Shamsuddin, 2002; Singh and Agarwal, 2005; Somasundar et al., 2005) or as an inhibitor of renal stone formation (Grases et al., 2000). It is important to view dietary phytate's positive and negative roles in human nutrition and health on a case-by-case basis. Phytate's negative roles tend to be most important in the developing world and in growth and development, whereas its positive roles are most important in the developed world and in health issues associated with aging.

Phytate in animal nutrition affects nutrient bioavailability and in areas of intense animal production also affects the environment. A side effect of the limited digestibility of phytate by monogastric animals is that the majority of P in feed made from cereal or a legume seed is excreted. For example, 80% of the P in a typical maize (*Zea mays* L.) diet fed to swine and poultry is in the form of phytate. The P is unavailable to these animals because they lack the phytases necessary to hydrolyze phytate (Ertl et al., 1998). Animal waste is an important source of P pollution in agricultural systems (Ertl et al., 1998). P pollutes watersheds via runoff and erosion of agricultural lands and causes eutrophication of streams, rivers, and lakes. This form of pollution reduces value and use of surface water for fisheries, recreation, industry, and drinking (Sharpley et al., 1994). Lott et al. (2000) estimate that 4100 million megagrams of field crops, fruits, and seeds are produced annually which contain 35 million megagrams of phytic acid, 9.9 million megagrams of which are phosphorus. Lott et al. (2000) used these estimates in conjunction with the FAO fertilizer-use data to calculate that the amount of phytic acid P produced by crops annually represents a sum equivalent to about 65% of the P fertilizer sold worldwide.

Ways to Reduce Phytic Acid in Foods

There are a number of approaches that can be taken to evaluate or deal with the effects of seed-derived dietary phytic acid. First, supplementation with minerals for humans can make up for the loss of minerals due to interaction with dietary phytic acid (Mendoza et al., 2001). For animal feed, a form of available P and minerals can be added (Baxter et al., 2003). For both food and feed, phytase enzyme can be added to break down the phytic acid, making minerals and P available. Plants can be genetically engineered to express high levels of phytase in the appropriate tissues of seeds, blocking phytic acid accumulation and resulting in seeds with high levels of residual phytase (Bilyeu et al., 2008). Food processing and preparation techniques, including thermal processing, mechanical processing, soaking and fermentation have been shown to decrease phytate levels (Hotz and Gibson, 2007). The approach reviewed here will be on using plant genetics to reduce seed phytic acid levels in major crop plants.

Development of Low Phytate Lines

Low phytic acid (*lpa*) mutants have been identified via phenotypic screens of mutagenized plants. The first two maize *lpa* mutants were isolated via a direct test for reduced seed phytic acid, using high voltage paper electrophoresis (Raboy et al., 2000). However, before the recent development of rapid and accurate single-seed tests for phytic acid (Gao et al., 2007), screening for phytic acid levels directly was time consuming. The screen that has largely been employed is for

the "high inorganic P" (HIP) phenotype typical of *lpa* seeds. The development of the quick and simple screen for relative seed inorganic P levels on a single-seed basis allowed for the effective and efficient screening of large populations of mutagenized plants. The method is based on the observation that reductions of phytic acid in the seed are frequently matched by increased seed inorganic P. Therefore, the identification of seed with high Pi can point to seed with low phytic acid. The HIP screen has subsequently been used to isolate additional *lpa* mutants in maize (Pilu et al., 2003; Raboy et al., 2001; Shi et al., 2005) and *lpa* mutants in soybean [*Glycine max* (L.) Merr.; Yuan et al., 2007; Wilcox et al., 2000], rice (*Oryza sativa* L.; Kim et al., 2008b; Larson et al., 2000; Liu et al., 2007b), barley (*Hordeum vulgare* L.; Larson et al., 1998; Rasmussen and Hatzack, 1998), wheat (*Triticum aestivum* L.; Guttieri et al., 2004) and work is in progress with other crops such as field pea (*Pisum sativum* L.), and common bean (*Phaseolus vulgaris* L.). Here we will first briefly describe a selection of crop *lpa* genotypes (Table 8-1) and review agromomic and nutrition studies conducted with them.

Biochemical Pathways to Phytic Acid; A Brief Background

One objective of isolating *lpa* mutations was "forward genetics" to understand the function and role of genes important to phytic acid synthesis. A brief review of the synthesis and storage of phytic acid will provide the necessary background. The biosynthesis of seed phytic acid can be thought of as consisting of three components: the synthesis or supply of the two basic substrates inorganic P (P_i) and the six carbon ring that serves as phytic acid's backbone, *myo*-inositol (Ins; Fig. 8-1 top); their conversion, via sequential phosphorylation of Ins or Ins(3) P_1 to phytic acid (Fig. 8-1 middle); and storage of phytic acid as a mixed salt of several nutritionally important mineral cations (Fig. 8-1 bottom).

The supply of P is a function of processes important to P uptake by the parent plant and transport to the developing seed. Very little progress has been made in the genetics of components of P transport processes specifically important to seed total P or phytic acid P. The synthesis of Ins is catalyzed by the enzyme *D*-*myo*-inositol-3-phosphate synthase, often referred to as "MIPS," which converts glucose 6-P to a specific Ins monophosphate, Ins(3) P_1 , which has a single P ester at the "D-3" position (Fig. 8-1). Ins(3) P_1 is then either phosphorylated directly to phytic acid, or first broken down to Ins and P_i via the action of Ins monophosphatase. In one possible pathway to phytic acid, the "lipid-independent pathway," either Ins or Ins(3) P_1 are then converted to phytic acid via the activity of a series of Ins and Ins phosphate kinases. In a second, alternative pathway, the "lipid-dependent pathway," Ins is first converted to phosphatidylinositol (PtdIns), which is then phosphorylated to PtdIns(4,5) P_2 , which is hydrolyzed to yield Ins(1,4,5) P_3 , and which finally is then converted to phytic acid. Most of the phytic acid synthesized in seeds is ultimately stored within specialized protein storage vacuoles, as mixed salts found within inclusions referred to as globoids. Transport functions are therefore important to phytic acid storage and may be important to components of its synthesis as well.

Maize *lpa* Genotypes

Three *lpa* genotypes of maize have been isolated and studied extensively. The first *lpa* allele identified in any crop was maize *lpa1-1* (indicating that it was the first recessive allele of the first *lpa* locus in maize; Raboy et al., 2000). Maize

Table 8-1. Low phytic acid loci of cereal and legume crops.†

Species	Locus	Map position	Seed phosphorus, Ins, and Ins phosphate phenotype
Maize	<i>lpa1</i>	1S	Allele-specific, variable reductions in PA, 50% to >90%, matched by increased P_i . Increased Ins.
	<i>lpa2</i>	1S	~50% reduced PA, matched by increased P_i and increased "lower" Ins phosphates such as Ins P_4 and Ins P_5 . Increased Ins.
	<i>lpa3</i>	1S	Largely embryo-specific ~50% reduced PA matched by increased P_i . Increased Ins.
Barley	<i>lpa1</i>	2H	Aleuone/Endosperm-specific ~50% reduced PA, matched by increased P_i . Reduced ~15% seed total P.
	<i>lpa2</i>	7H	~50% reduced PA, matched by increased P_i and increased "lower" Ins phosphates such as Ins P_4 and Ins P_5 . Increased Ins.
	<i>lpa3</i>	1H	~70% reduced PA, matched by increased P_i . Increased Ins.
	M640	7H	Aleuone/Endosperm-specific ~50% reduced PA, matched by increased P_i . Reduced ~15% seed total P.
	M593	4H	~50% reduced PA. Other linked mutants, all possibly alleles of MIPS gene, have PA reductions from ~10% to >90%.
Rice	M955	1H	> 90% reduced PA, matched by increased P_i . Increased Ins.
	<i>lpa1</i>	2	~40% reduced PA, matched by increased P_i .
	<i>lpaN15-186</i>	3	~75% reduced PA, matched by increased P_i .
	<i>lpa-XS110-1</i>	3	~65% reduced PA, matched by increased P_i .
Soybean	<i>pha1</i>	LG N	~80% reduced PA, matched by increased P_i , when plants homozygous for both <i>pha1</i> and <i>pha2</i> . Reduced field emergence enhanced if seed produced in tropical environment.
	<i>pha2</i>	LG L	~80% reduced PA, matched by increased P_i , when plants homozygous for both <i>pha1</i> and <i>pha2</i> . Reduced field emergence enhanced if seed produced in tropical environment.
	<i>lpa-ZC-2</i>	LGB2	~50% reduced PA, matched by increased P_i and increased "lower" Ins phosphates. Less effect on field emergence than observed for other soybean <i>lpa</i> mutations.
	LR 33-MIPS	Not Known	Ins decreased 60% to 80%. PA decreased ~50%. Reduced field emergence enhanced if seed produced in tropical environment.

† Please see the following references for each species. Maize: Raboy et al. (2000); Shi et al. (2003, 2005, 2007). Barley: Larson et al. (1998); Hu and Raboy, unpublished data (2008); Roslinsky et al. (2007). Rice: Larson et al. (2000); Liu et al. (2007b); Kim et al. (2008a, 2008b). Soybean: Oltmans et al. (2004); Walker et al. (2006); Wilcox et al. (2000); Yuan et al. (2007).

lines homozygous for *lpa1-1* exhibit a 66% reduction in seed phytic acid P, as compared with sibling nonmutant seeds and molar equivalent increase in P_i with no significant change in seed total P.

The gene perturbed in maize *lpa1-1* was identified as a "multidrug resistance-associated protein," maize MRP4, which is an ATP binding cassette (ABC) transporter containing 11 exons (Shi et al., 2007). While the function of the ABC transporter has not yet been determined, it may be involved in the transport of phytic acid from its site of synthesis to its storage site in protein storage vacuoles.

The isolation of maize *lpa1* and its subsequent identification as an ABC transporter represents the first breakthrough in understanding transport processes important to seed phytic acid.

The second *lpa* locus in maize was designated *lpa2*. Interestingly, the first *lpa2* recessive allele isolated in the genetic screens was not a result of mutagenesis but was a preexisting, naturally occurring allele (Raboy et al., 2000). Lines homozygous for *lpa2-1* exhibit 50% reduction in seed phytic acid P along with elevated levels of Pi, Ins, and Ins phosphates with five or fewer P esters (whereas phytic acid has six P esters per molecule). *Lpa 2* encodes an Ins(1,3,4)P₃ 5-/6-kinase which catalyzes the conversion of Ins trisphosphate intermediates to Ins P₅, one step in the pathway to Ins P₆ (Shi et al., 2003; Fig. 1). Knocking out the function of this gene resulted in a 30% reduction of phytic acid in the seed, suggesting that phytic acid is produced by multiple pathways in maize (Shi et al., 2003).

Lines homozygous for recessive alleles of a third maize *lpa* gene, *lpa 3*, exhibited a 50% reduction in phytic acid and elevated Pi and Ins. The gene encodes an Ins kinase that is expressed in embryos. Like maize *lpa1*, the isolation of maize *lpa3* and determination that it encodes an Ins kinase represented an important breakthrough in understanding the phytic acid pathways in seeds. Historically, there had been evidence that there are two possible pathways from Ins to phytic acid; one that entirely consisted of the sequential phosphorylation of Ins and soluble Ins phosphates (the "lipid-independent" pathway) and one that involved in part phosphatidylinositol phosphate intermediates (the "lipid-dependent" pathway) (Fig. 8-1). Analyses of *Arabidopsis* Ins phosphate kinase mutants indicated a role for the lipid-dependent pathway (Stevenson-Paulik et al., 2005). Demonstration that knocking out the function of an Ins kinase (maize *lpa3*) partially blocked phytic acid synthesis indicates that the lipid-independent pathway plays a part in phytic acid synthesis, but it also suggests either the presence of other genes with similar function or the existence of two distinct phytic acid synthesis pathways (Shi et al., 2005).

Barley *lpa* Genotypes

Screening M₂ lines derived from the barley cv. Harrington for high seed inorganic P resulted in the identification of 24 *lpa* mutants, representing alleles at a minimum of six *lpa* loci (Hu and Raboy, unpublished data, 2008). This represents the greatest number of *lpa* loci identified in a single species (previously, the maximum was four loci in soybean; Table 8-1). Screening for mutations in the barley cv. Pallas also revealed a high frequency of mutation and identified nine *lpa* mutants at two distinct loci (Rasmussen and Hatzack 1998). The allelic relationship between these two loci and those described above has not been determined.

Four of the mutants isolated in the cv. Harrington background (M422, M1070, M635, and M955) were chosen for initial study since their phenotypes appeared to represent the full spectrum of phenotypes observed in the mutant collection. Each of the four barley *lpa* mutants segregates as recessive alleles of single genes (Larson et al., 1998; Dorsch et al., 2003). Following chromosomal mapping, M422 (chromosome 2) was designated barley *lpa1*, M 1070 (chromosome 7) as barley *lpa2*, and M 635 (chromosome 1) as barley *lpa3* (Larson et al., 1998; Roslinsky et al., 2007). M955 is linked to *lpa3* on chromosome 1 but represents a nonallelic gene. More recent work (Hu and Raboy, unpublished data, 2008) has shown that a fifth mutant, M 640, represents a fifth gene that is linked to but nonallelic to *lpa2* on

chromosome 7. This recent work has also shown that five of the initial 24 mutants (M593, M678, M889, M1572, and M1954) represent mutations linked to the barley genome's single MIPS-encoding gene on chromosome 4. These mutants condition reductions in seed phytic acid ranging from 10 to 90%. However, to date none of the genes perturbed in the *lpa* barley mutants have been definitively identified.

Homozygosity for barley *lpa1* was shown to result in a 50% reduction in seed phytic acid matched by molar equivalent increases in Pi (Larson et al., 1998). Further studies revealed that the reduction in phytic acid is endosperm/aleurone-specific (barley *lpa1* embryos have wild-type or >wild-type levels of phytic acid) and that recessive alleles of barley *lpa1* also result in a reduction in seed total P of ~15% (Ockenden et al., 2004; Hu and Raboy, unpublished data, 2008).

Homozygosity for barley *lpa 2-1* results in a 35 to 50% reduction in seed phytic acid accompanied by increases in P_i as well as increases in Ins and Ins phosphates with less than six phosphate esters per molecule, a phenotype similar to maize *lpa2-1* (Dorsch et al., 2003). Homozygosity for barley *lpa3-1* results in a 75% reduction in phytic acid and increased Pi and Ins. Homozygosity for M955 results in the largest reduction in phytic acid of any crop genotype that is viable, with reductions of ~90%, accompanied by matching increases in Pi and Ins (Dorsch et al., 2003).

As indicated above, in the seed produced by maize *lpa2* and *lpa3*, as well as barley *lpa2*, *lpa3*, and M955, all *lpa* mutations that perturb the conversion of Ins to Ins P_6 but do not perturb Ins synthesis (as in MIPS mutants such as soybean LR33, see below) also have elevated seed Ins, probably because of the reduced sink for Ins during seed development. Karner et al. (2004) demonstrated that this elevated seed Ins has downstream effects on pathways that use Ins and thus also results in elevated raffinose and elevated sucrose. Conversely, mutations that reduce seed phytic acid by blocking Ins synthesis also result in reduced raffinose (Hitz et al., 2002). This illustrates that since *lpa* mutations affect important metabolic pools in seeds, they may have downstream impacts on pathways and compounds other than P and phytic acid.

Wheat *lpa* Genotypes

The wheat breeding line A95631S-Js-12 was mutagenized with 2% (v/v) EMS and seed from 562 M_1 were planted, from which 2000 M_2 plants were screened for high inorganic phosphorus. A single plant (Js-12-LPA) was identified as an *lpa* mutant. The line has 37% reduction in seed phytic acid and a 5-fold increase in P_i , although total P levels were the same between the wild-type and mutant line. The mutation appears to be controlled by two genes (Guttieri et al., 2004).

Rice *lpa* Genotypes

A single low phytic acid rice mutant was identified via screening M_2 plants of cv. Kaybonnet gamma irradiated seeds. The rice mutant (*lpa1*) is caused by a single recessive allele which reduces seed phytic acid by about 45% accompanied by molar equivalent increase in Pi (Larson et al., 2000). The rice *lpa1* gene was recently shown to encode a sequence homologous to a gene encoding 2-phosphoglycerate kinase (2-PGA kinase) in hyperthermophilic methanogens, members of the prokaryotic Archaea (Kim et al., 2008a). 2-PGA kinase converts 2-PGA to 2,3-bisPGA. However, the biochemical function of the protein encoded by rice *lpa1*, and its role in seed phytic acid synthesis, has not yet been determined.

In a separate study, six Chinese rice cultivars representing both *indica* and *japonica* subspecies were gamma-irradiated, and in some cases additionally treated with sodium azide, and screened for high seed inorganic P (Liu et al., 2007b). Five nonlethal *lpa* mutants, each inherited as a single-gene recessive allele, were identified. Mapping and allelism tests indicated that these represent four loci, one linked to rice *lpa1* and a second mapping to a site syntenic with maize *lpa3*.

Soybean *lpa* Genotypes

A mutation in the MIPS gene displaying seed-specific expression was identified via screening soybean lines derived following chemical mutagenesis (Hitz et al., 2002). Along with 50% or greater reductions in seed phytic acid, this mutant line, LR33, also has reduced Ins and raffinose saccharides.

M₃ seed derived from the soybean breeding line CX1515-4 were screened for high inorganic P and two mutants were identified, M153 and M766. M153 exhibited approximately 80% reduction in phytic acid and molar equivalent increases in Pi. M766 had 40% less phytic acid and also showed molar equivalent increases in Pi (Wilcox et al., 2000). *Lpa* lines derived from M153 exhibit digenic inheritance and the genes have been designated *pha1* and *pha2* (Oltmans et al., 2004). The loci map to linkage group L and linkage group N and explain 11 and 41% of phenotypic variation for seed phytic acid, respectively. An epistatic interaction between the two loci accounts for an additional ~10% of the variation for this trait (Walker et al., 2006). Independent screening of soybean M₂ seed identified at least one additional soybean *lpa* locus, represented by the *lpa*-ZC-2 mutation (Yuan et al., 2007).

Genetic Engineering of the Low-Phytate Trait

Genetic engineering approaches to altering seed phytate levels may provide some advantages to the use of "whole-plant" gene mutations described above. One possible advantage is that via genetic engineering one can target the block in phytic acid synthesis or accumulation to a specific tissue of the seed, thus avoiding negative impacts on whole-plant function that result from a mutation in the phytic acid pathways (see below). For example, the maize *lpa1*/ABC transporter was used in an engineering approach to target or restrict the block in phytic acid accumulation to the maize embryo or the developing soybean seed, yielding the low-phytate trait with minimal impacts on yield or plant performance (Shi et al., 2007). Another potential advantage made possible by genetic engineering is the ability to introduce genes encoding stable phytases that are active both during seed development and when mature seeds are subsequently consumed in foods or feeds. Transformation of soybean with a bacterial phytase whose expression was targeted to the cytoplasm of developing seed cells reduced mature seed phytic acid levels by more than 90% (Bilyeu et al., 2008). It also produced seeds with elevated endogenous phytase levels that when consumed in a diet would act on phytates consumed from other components of the diet or feed, such as those contributed by the maize component.

Agronomic Studies of *lpa* Genotypes

The agronomic performance of low-phytate crop lines has been shown to vary among crops, and between genes, alleles, and environmental conditions. Isolines of barley that were either homozygous wild type or homozygous for *lpa1*-1, *lpa2*-1, or *lpa3*-1 had similar yields when grown in relatively nonstressful

production environments that utilized irrigation (Bregitzer and Raboy, 2006). In these environments, M955, with greater than 90% reduction in seed phytate, still had 13% less yield than its wild-type isolate. In more stressful, nonirrigated production environments, only *lpa1-1* displayed yields similar to wild type, whereas *lpa2-1* and *lpa3-1* each had 25% reduction in seed yield, and M955 had nearly 35% reduction in seed yield. Thus, reductions in yield were proportional to reductions in seed phytic acid, were more pronounced in more stressful production environments, and were minimal only in one genotype (barley *lpa1-1*). Barley *lpa1-1* differs from the other three genotypes in that its recessive alleles result in a localized, tissue-specific block in phytic acid accumulation. More recent work has shown that barley M640 is phenotypically similar to barley *lpa1-1* in that it also has an aleurone-specific impact on phytic acid (Hu and Raboy, unpublished data, 2008), and yield trials indicate that a low-phytate barley cultivar produced from M640 has yields comparable with conventional cultivars in a wide variety of production environments (Bregitzer et al., 2008). Thus, conventional genetics and breeding approaches can achieve at least one of the advantages provided by genetic engineering described above: genotypes in which blocks in the phytic acid pathways are restricted to specific tissues of the seed, such as the embryo or aleurone layer, thus minimizing the negative impact on whole plant performance and yield.

Germination and field emergence is reduced in many but not all soybean *lpa* genotypes. When seeds homozygous for recessive (*mips*) alleles of the soybean MIPS-encoding gene (the LR33 mutations) were produced in a tropical environment, subsequent field emergence was greatly reduced (8%), as compared with the field emergence rate of seeds of the same genotype produced in a temperate region (63%), or of wild-type seeds produced in either a tropical or temperate environment (77 and 83%, respectively; Meis et al., 2003). Why a MIPS mutation has a greater impact on field emergence of seeds when produced in a tropical versus temperate environment is unknown at present. Reduced emergence of about 20% was also observed in low-phytate soybean carrying the *pha1* and *pha2* alleles, and emergence was negatively correlated with seed Pi ($r = -0.81$; Olthmans et al., 2005), but there were no differences in seed yield, maturity, protein, oil, height, and lodging (Hulke et al., 2004). Like the observation with the *mips* lines, reduction in field emergence of *pha1::pha2* soybean isolines was more pronounced for seeds produced in subtropical Puerto Rico than in temperate Iowa (Anderson and Fehr, 2008). In the Puerto Rico field tests, emergence rate was only 10% of wild type when the tested seeds were produced in a July planting, but when the tested seeds were produced in a May planting, emergence rate was 60% of wild type. Perhaps this larger effect on field emergence is attributable to higher temperatures and humidity during seed filling.

Initial yield trials with the first-generation wheat low-phytate lines provided results that are in contrast to the inverse relationship between stress and productivity of low-phytate genotypes described above (Guttieri et al., 2006a). In the irrigated (less stressful) production environment of Aberdeen, ID, low-phytate wheat in both hard red and soft white genetic backgrounds had grain yield reductions up to 25% as compared with normal phytate lines. However, under nonirrigated (more stressful) conditions of Tetonia, ID, where wild-type yield was only 35% of that in the irrigated conditions; there was no reduction in yield in *lpa* lines compared to the wild type.

Studies with soybean and barley *lpa* genotypes illustrate the potential of classical genetics and breeding approaches for overcoming agronomic problems associated with the *lpa* trait. Selection within lines segregating for a given *lpa* allele can identify lines with enhanced agronomic performance (Oltmans et al., 2005). Identification of genes which if perturbed have little impact on agronomic performance, such as barley *lpa1* and M640 (Bregitzer and Raboy, 2006; Bregitzer et al., 2008; Hu and Raboy, unpublished data, 2008) or soybean *lpa*-ZC-2 (Yuan et al., 2007), can lead to low-phytate cultivars with superior agronomic performance.

Amount and Distribution of Phosphorus and Minerals in Seed of *lpa* Crops

Since phytate deposits represent a large fraction of P and minerals such as K and Mg in the cereal grain and since these deposits are localized to the germ and aleurone layer of the mature grain, it is possible that mutations that perturb phytic acid synthesis or storage may alter the distribution of P and minerals in the cereal grain by shifting some portion of the seed P and mineral stores from the outer tissues of the germ and aleurone layer to the central endosperm. This may enhance the nutritional value of milled products like white rice or wheat flour, which largely consist of central endosperm. The rice *lpa1-1* mutation influences the phytic acid content of the embryo (58% reduction as compared with wild type) more than the aleurone layer (43% reduction; Liu et al., 2004). There were increases in Mg, Ca, and Mn in the *lpa1-1* embryo and decreases in Mg and Mn in the "rest-of-grain" fraction consisting of endosperm and aleurone layer. Analyses of milling fractions obtained from wild type and *lpa1-1* rice indicated elevated total P, K, and Mg (25 to -40%) in *lpa1-1* milled rice compared with wild type (Bryant et al., 2005). These changes in the distribution of P and minerals in the tissues of the rice grain are not associated with significant changes in the total levels of these constituents in whole grains. Analyses of a mutation allelic to rice *lpa 1-1*, *HIP1*, showed that it reduced phytic acid P and increased K, Ca, Zn, Mg, Fe, and Cu in milled rice, as compared with wild type. These results showed the potential increase in the amount of Fe, Zn, and Ca in the edible part of the rice grains and thus provide an important added value to the *lpa* mutation (Ren et al., 2007).

A set of barley isolines consisting of wild type and four *lpa* mutations that reduce seed phytate from ~40 to >90% represents an excellent experimental model to test the hypothesis that mutations that block phytic acid may greatly alter grain mineral distribution. In contrast to the work with rice *lpa1-1*, analyses of milled fractions obtained from the four barley *lpa* isolines revealed no large effects on mineral distribution (Liu et al., 2007a). Additional analyses of *lpa* genotypes in barley, maize, and wheat have described changes in the number and size of globoids, and significant alterations in the distribution of P, but often only minor changes in the distribution of minerals (Guttieri et al., 2006b; Joyce et al., 2005; Ockenden et al., 2004). Thus, the distribution of minerals in the mature cereal grain may be largely independent of phytic acid synthesis or its localization. However, end-use beneficial allele- or gene-specific shifts in mineral distribution may occur.

In analyses reported to date, mutations in all but one *lpa* gene have little or no impact on seed total P. The one exception is barley *lpa1*. Eight recessive alleles of barley *lpa1* have been isolated and all appear to reduce whole seed total P by

~15% (Ockenden et al., 2004; Hu and Raboy, unpublished data, 2008). Reduced seed total P is an outcome of an endosperm/aleurone-specific reduction in total P; *lpa1-1* germs have elevated total P but the net effect is reduced whole seed total P. This effect of barley *lpa1* is in contrast to the effect of other *lpa* mutations, which often result in elevated endosperm total P (Guttieri et al., 2006b). Reduced seed total P could be a trait valuable for managing P and reducing waste P in ruminant production (Volk et al., 2000; Warden and Russell, 2004) and possibly of value in other end-uses such as in reducing P levels in "Distiller's Dry Grains," a side-product following milling of grains for biofuel production (Maguire et al., 2007).

Animal Nutrition Studies

Improved Phosphorus Digestibility

Low-phytate crops have the potential to address two major management concerns in animal production: providing adequate available P for optimum animal productivity and minimizing animal waste P. First, nonruminant, monogastric animals including swine and poultry require modifications in grain diets because most of the P in the grain is stored as phytic acid, which monogastrics do not utilize efficiently; thus, only ~25% ($\pm 10\%$) of seed total P is available (Cromwell et al., 1993). Therefore, the production of healthy animals requires diet additives such as monosodium P or phytase to address the limited P availability (Yan et al., 2003). Phosphate supplementation adds cost to production as well as cost to the environment, which is the second area where low phytic acid crops have potential value. Since only a small percentage of P is used by the animal, a large portion is excreted, which can contribute to P pollution in the form of eutrophication of surface waters (Ertl et al., 1998). Worldwide use of P fertilizer (P_2O_5) is on the rise and has increased from 10×10^6 to 30×10^6 Mg from 1960 to 2000 (Tilman et al., 2001). As a result of the intensification of animal production in regions of the southeast and northwest USA, 11% of counties have a manure P surplus (Maguire et al., 2007).

This contrasts with the broader concern that P is one of the most limiting nutrients in many agriculture systems and is a finite nonrenewable resource. It is estimated that there is enough rock phosphate to last until 2070 with the current rate of extraction (Steen, 1998). Substitution of normal feed grains with low-phytate grains can both increase the available P for nonruminants and decrease waste P (see below). Phytase supplementation, commonly using fungal phytase, hydrolyses phytic acid (Vats et al., 2005), is effective in increasing available P and reducing P in excreta (Baxter et al., 2003; Powers et al., 2006). The addition of phytase is also used to improve the feed quality of maize (Cowieson, 2005). Therefore, phytase supplementation, or the use of low-phytate crops or a combination of the two, offers opportunities to use precious P resources more conservatively. A study of P in animal production in the USA found that animal diet modification, such as reducing added P in diet or adding phytase, can significantly reduce P pollution potential (Maguire et al., 2007).

Numerous animal feeding trials have been conducted to determine how the addition of low-phytate grain to diets affects P use and animal nutrition (Table 8-2). The major finding is that the substitution of standard, "normal phytate" grain with low-phytate grain improves P digestibility. The first such study (Ertl et

Table 8-2. Published human and animal nutrition studies that evaluated low-phytate crop genotypes.

Crop species	LPA line	Test organism	Citations	
Maize	<i>lpa1</i>	human	Mendoza et al. (1998, 2001); Adams et al. (2002); Hambidge et al. (2004, 2005); Mazariegos et al. (2006)	
		swine	Spencer et al. (2000a, 2000b); Veum et al. (2001); Baxter et al. (2003)	
		poultry	Ertl et al. (1998); Douglas et al. (2000); Li et al. (2000); Peter and Baker (2002); Ceylan et al. (2003); Miles et al. (2003); Jang et al. (2003); Yan et al. (2003)	
	Nutradense	trout	Sugiura et al. (1999)	
		human	Hambidge et al. (2004)	
		swine	Hastad et al. (2005)	
	Other/undefined <i>lpa</i> source	poultry	Klunzinger et al. (2005)	
		swine	Wienhold and Miller (2004)	
		poultry	Adeola et al. (2005)	
	Barley	<i>lpa1-1</i>	swine	Veum et al. (2002); Thacker et al. (2003, 2004); Leytem et al. (2004); Veum et al. (2007); Htoo et al. (2007a, 2007b)
poultry			Jang et al. (2003); Li et al. (2001); Leytem et al. (2007); Salarmoni et al. (2008)	
			trout	Sugiura et al. (1999); Overturf et al. (2003)
<i>lpa3-1</i>		swine	Thacker et al. (2003, 2004); Leytem et al. (2004); Veum et al. (2007); Htoo et al. (2007a, 2007b)	
		poultry	Leytem et al. (2007); Salarmoni et al. (2008)	
			trout	Overturf et al. (2003)
<i>lpa2-1</i>		poultry	Jang et al. (2003); Salarmoni et al. (2008)	
		M955	swine	Thacker et al. (2003); Leytem et al. (2004); Veum et al. (2007); Htoo et al. (2007a, 2007b)
			poultry	Linares et al. (2007); Leytem et al. (2007); Salarmoni et al. (2008)
sheep			Leytem et al. (2007)	
Soybean		PLPA and PLPB	trout	Overturf et al. (2003)
		Undefined <i>Lpa</i> source	rat	Poulsen et al. (2001)
			swine	Dilger and Adeola (2006a)
	CX1384-1	poultry	Sands et al. (2003); Adeola et al. (2005); Karr-Lilienthal et al. (2005); Dilger and Adeola (2006b)	
		canine	Yamka et al. (2005, 2006)	
		poultry	Powers et al. (2006)	

al., 1998) evaluated grain produced by two maize isohybrids when used in chick feeds. Isohybrids are produced with pairs of isolines and are similar to isolines in that they are genetically similar to each other in essentially every way except for differences in alleles at one or a few genes. Ertl et al. (1998) used isohybrids that were either "normal phytate," which produced grain with 3.8 mg total P g⁻¹ dry weight and 3.2 mg g⁻¹ phytic acid P or *lpa1-1*, which produced grain with 3.9 mg total P g⁻¹ and 1.3 mg phytic acid P g⁻¹. If phytate P is largely nonavailable to non-ruminants, and all other forms of seed P are available, then the wild-type grain is estimated to contain about 0.6 mg available P g⁻¹, or 16% of grain total P, and the *lpa1-1* grain is estimated to contain 2.6 mg g⁻¹ available P g⁻¹, or 67% of grain total P. The two maize grain types differed primarily only in their P chemistry and the formulated diets were similar in all ways except for P chemistry. The chick feeding study yielded measures of P availability for grain produced by the wild-type isohybrid ranging from 30 to 48%, whereas P availability for the maize *lpa1-1* grain ranged from 70 to 91%. Thus, the measures of P availability obtained from the animal trial paralleled the estimates of P availability on the basis of analysis

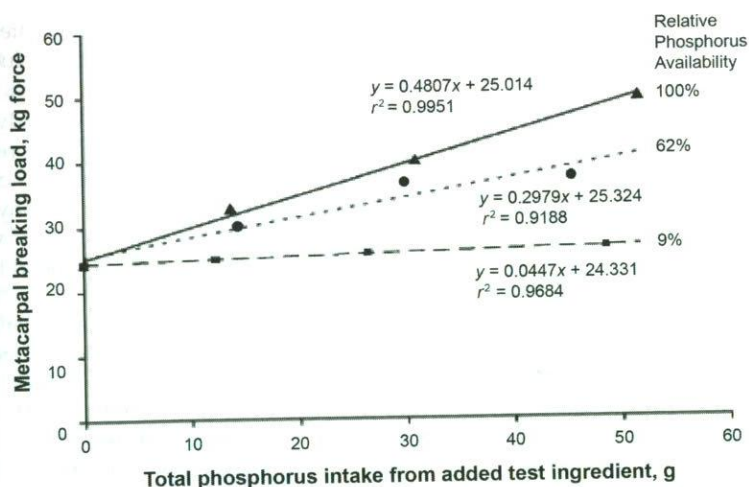


Fig. 8-2. Phosphorus bioavailability in pigs fed diets developed from normal phytate and low phytic acid corn (*lpa1-1*) (from Spencer et al., 2000a). Estimation of relative P availability for low-phytate (LP; *lpa1-1*) and normal maize using bone breaking load of the fourth metacarpal as the criterion. Points are treatment means with five pigs/treatment. Normal maize (■), low-phytate corn (●) and monosodium phosphate (▲).

of seed P chemistry. Fecal P of birds consuming *lpa1-1* maize diets was reduced by 9 to 40% as compared with fecal P of birds consuming wild-type maize diets (Ertl et al., 1998). Similar benefits of low-phytate maize were also observed in additional, independent chick studies. Douglas et al. (2000) observed P to be 2- to 3-times more available in *lpa* than conventional maize diets. A study of 150 male chicks fed maize as their only phytate source showed that those chicks fed the low-phytate maize (*lpa 1-1*) had 18% higher P retention than those chicks fed the wild-type corn (Li et al., 2000).

Spencer et al. (2000a) evaluated grains produced by these same maize isohybrid when used in pig feeds. Those animals fed diets containing *lpa 1-1* maize and no added P had P digestibility of 48%, whereas animal receiving the diet formulated with wild-type maize and no additional P, had 17% P digestibility (Fig. 8-2) (Spencer et al., 2000a). Use of the *lpa1-1* maize did not cause negative effects on pig performance, bone strength, or carcass characteristics (Spencer et al., 2000b). In contrast, consumption of *lpa1-1* grain resulted in carcasses with reduced backfat and a higher percentage of "lean" meat. Subsequently, it was shown that use of low-phytate maize as a substitute for normal maize can reduce cholesterol in eggs (Stilborn et al., 2002). Thus, consumption of low-phytate grains might enhance product quality in addition to enhancing the management of P. The nutritional mechanism leading to this unanticipated benefit, reduced fat and cholesterol, is not known at present.

Low-phytate soybean had 12 to 16% more bioavailable P when fed to broiler chicks as compared to normal-phytate soybean, as measured over a 14-d feeding trial (Sands et al., 2003). Soybean meal prepared with low-phytate soybean had greater P availability for poultry than soybean meal heated in an extractor for 45, 60, or 90 min. Low-phytate meal had nearly 50% higher P availability than normal

soybean meal. The weight gain to feed ratio of chicks was about 24% greater for chicks fed basal diet plus 8% low-phytate soybean meal versus basal diet plus 9% soybean meal treated in an extractor for 45 min (Karr-Lilienthal et al., 2005).

Low-phytate barley has proved to be an especially effective tool to study the role of phytate in animal nutrition because numerous *lpa* lines exist with reductions in phytic acid ranging from 35 to more than 90%. In a study using 25 cross bred barrows (pigs), P digestibility increased linearly ($r^2 = 0.64$) with nonphytate phosphorus when the pigs were fed barley with phytate content of 68% of wild type (*lpa1-1* or "M422"), 41% of wild type (*lpa3-1* or "M635"), and 3% of wild type (M955) (Thacker et al., 2003). A similar study utilizing pigs (Veum et al., 2007) and the same four barley lines found similar results concerning the inverse relationship between grain phytate P and P retention. The low-phytate, near isogenic lines of the barley cv. Harrington, *lpa1-1*, *lpa3-1*, and M955, used in the study contained 47, 66, and 80% less phytic acid, respectively, than normal barley. As the percent phytate in the diet decreased, the percent retained P increased. Pigs fed wild type, *lpa1-1*(M422), *lpa3-1*(M635), and M955 displayed percent retained P of 51.4, 58.7, 60.1, and 66%, respectively, and percent P excreted of 48.6, 41.3, 39.9, and 34%, respectively (Veum et al., 2007).

A study of grower pigs fed "hulled" and "hull-less" barleys that were either normal or low-phytate identified differences in P retention and excretion on the basis of P composition of the diet such that P excretion was reduced by 35% when pigs were fed low-phytate barley lines which had a 31% reduction in phytic acid. The amount of P retained in the body was 49 and 65% for normal and low phytate barley, respectively (Htoo et al., 2007a).

In another study (Li et al., 2001), turkey poults were fed barley diets of similar composition except that one was produced from normal barley and contained 0.3% available P (nonphytate P) and one was composed of low-phytate barley (*lpa1-1*) and had 0.36% available P. It was found that those turkey fed diets containing *lpa* barley retained 11% more P and excreted 41% less P. There was no compromise in turkey health as measured by weight gain and tibia and toe ash. Poults fed a third diet, developed from normal barley but formulated to contain 0.36% available P, similar to the *lpa1-1* diet, had less P retention than those poults fed *lpa* barley. This study illustrated the potential value of *lpa* grains in animal feeds; it reduces the need for added P as well as reducing P excretion.

Another potential benefit from the use of low-phytate grains in feeds is that the enhanced P availability and resulting reduced excretion shifts the ratio of P and N in the animal waste in a favorable manner. The true metabolizable energy was 8% greater for ducks fed low-phytate soybean meal as compared with normal soybean meal. The nitrogen retention was 25% greater in ducks fed the low-phytate soybean meal and nitrogen output was reduced (Adeola, 2005). Low-phytate barley improved pig manure quality for use as a fertilizer by increasing its nitrogen to phosphorus ratio by 40% (Htoo et al., 2007b). While *lpa* crops reduce total P in swine manure by 33%, it does not affect the solubility of P remaining in the manure. There is very little phytic acid in manure from wild-type or *lpa* barley, suggesting phytase activity in the animal hind gut (Leytem et al., 2004).

Low phytic acid crops have shown variability in their effect on gross energy of the feed. For example, the gross energy of barley *lpa3-1*(M635) and M955 was 6% lower than Harrington and *lpa1-1* M422 (Overturf et al., 2003). A study of poultry fed diets composed of low-phytate barleys *lpa1-1*(M422), *lpa2-1*(M1070),

lpa3-1(M635), and M955 (Salarzadeh et al., 2008) found that all chicks had similar feed intake and weight gain on each of the diets except for *lpa1-1*(M422), where there were decreases in weight gain of 16%. This difference was only observed at the standard dicalcium phosphate addition to the control of 1.36% and not at 0.78% added phosphate. These data suggest that individual low-phytate lines may affect feed intake and weight gain differently.

Improved Micronutrient Absorption

A number of studies have shown that the substitution in feeds of low-phytate grains for standard grains can enhance the availability and retention of nutritionally important mineral cations as well as P. These studies also provide a valuable animal model for assessing the impact of dietary phytic acid on mineral nutritional health in humans. One of the most commonly reported effects is an increase in calcium absorption by the animal. P nutritional status has an important impact on calcium status and on bone health. Ertl et al. (1998) reported that blood P and calcium levels in birds consuming *lpa1-1* maize diets was 46 and 49% greater, respectively, than that observed in birds consuming the wild-type maize diets, and this resulted in increases of 12, 10, and 13% in bone (tibia) ash, bone P, and bone calcium, respectively. This represents the first indication that altering the phytic acid and available P in a basal diet may have additional benefits beyond P nutrition.

Enhanced calcium nutrition was also reported in swine (Veum et al., 2001), additional poultry studies (Li et al., 2000), and trout (Overturf et al., 2003). Pigs fed diets prepared with maize *lpa1-1* had an increase in Ca absorbed/intake ratio, 74%, as compared with pigs fed wild-type maize, 67.2% (Veum et al., 2001). A linear, inverse relationship between dietary phytic acid and calcium was observed in young swine fed diets developed from near-isogenic barley lines barley that were either homozygous wild type, *lpa1-1*, *lpa3-1*, or M955. The calcium retained/intake ratio was 27.6 for animals fed normal-phytate, wild-type barley and increased to 31.7, 43.2, and 53.8 in animals fed *lpa1-1*, *lpa3-1*, or M955 barely (47, 66, and 80% less phytic acid, respectively; Veum et al., 2007). In chicks, a 7.2% increase in Ca absorption was observed with *lpa1-1* maize as compared with normal maize (Li et al., 2000).

The negative effect on zinc nutrition of the phytic acid level found in normal-phytate, standard grains, and the substantial improvement in zinc nutrition achieved via the use of a low-phytate grain, is well illustrated in the study of Linares et al. (2007). Chicks were fed diets consisting of M955 barley (>90% reduced phytic acid), or "normal phytate" standard barley, in each case either with no zinc supplementation, or with 10 or 20 mg kg⁻¹ zinc supplement. Overall, substitution of M955 for standard barley enhanced toe and tibia zinc by 46 and 25%, respectively. Chicks fed M955 with no additional zinc displayed a zinc nutritional status (as illustrated in Fig. 8–3 via tibia zinc levels) that was as good or better than that observed in chicks fed a diet consisting of normal-phytate barley with 20 mg kg⁻¹ supplemental zinc. In fact, the benefit of zinc supplementation was only observed in chicks fed normal-phytate barleys. Zinc nutritional status was uniformly high in chicks fed M955 barley, and zinc supplementation yielded no further enhancement. This result can be interpreted as indicating that in some cases the benefit of dietary zinc supplementation is only to overcome the negative effect of dietary phytic acid. This result has implications for the development of public health strategies to overcome zinc or iron deficiency in human populations

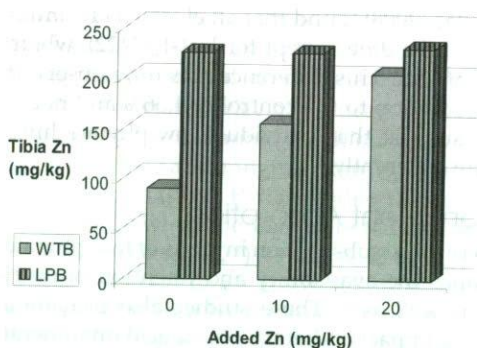


Fig. 8-3. Interaction of barley genotype [wild type (WTB) and low-phytate (LPB), M955] and supplemental Zn (0.0 mg/kg, 10 mg/kg or 20 mg/kg) on tibia Zn concentration in chicks at 21 d of age (from Linares et al., 2007).

that rely on cereals and legumes as staple foods. If in some important cases the benefit to supplementation is only to overcome the negative impact of dietary phytic acid; then the development and use of low-phytate crops may provide an effective alternative approach. Whereas most studies look at the effects of *lpa* feed on nonruminant animals, one study showed that sheep (ruminant) had improved total tract digestion of Fe, Mg, and Zn when fed *lpa* barley (Leytem et al., 2007).

The findings that low phytate crops improve micronutrient absorption in monogastric animals are important to animal production and may reduce the need for diet supplements. As mentioned above, these studies also serve as models that can be applied to human nutrition and indicate a value to micronutrient status in humans that may be possible by the addition of low-phytate crops to the diet.

Human Nutrition Studies

Studies with human subjects that used stable isotopes demonstrated that dietary phytic acid inhibits absorption of Fe, Zn, Ca, and Mg (Hurrell, 2003; Bohn et al., 2004). The use of foods developed from *lpa* crops provides an additional approach to studying the impact of dietary phytate on a human nutritional status. In vitro assays with human caco-2 (colon adenocarcinoma) cells are used to study intestinal absorption of nutrients (Phillippy, 2006). Using the Caco-2 cell assay, phytic acid reduced the uptake and transport of Fe and Zn (Han et al., 1994). Using the same Caco-2 assay to assay, Fe absorption from *lpa1-1* versus wild-type maize showed a 50% increase in Fe absorption from *lpa1-1* maize as compared with the wild-type isolate (Fig. 8-4; Raboy, 2007). The addition of ascorbic acid, which is a known enhancer of Fe absorption, increased Fe bioavailability in wild type to an extent, 40%, similar to the enhancement provided by the *lpa* gene, and also further enhanced Fe absorption from *lpa1-1* maize by 50% (Fig. 8-4). These results provide further evidence that the inherent nutritional value of *lpa* grain can provide benefits equal to those achieved via supplementation, whether as in this case it concerns Fe and ascorbic acid supplementation, or as discussed previously P nutrition and P supplements or zinc nutrition and zinc supplements.

As compared with the relatively large number of studies that evaluated low-phytate genotypes in animal models, only a small number of short-term, small-scale studies have been conducted with human subjects (Table 8-2). Iron absorption was significantly impacted by maize source in a study of 14 men consuming tortillas for breakfast for 2 d, prepared from either normal or *lpa1-1* maize.

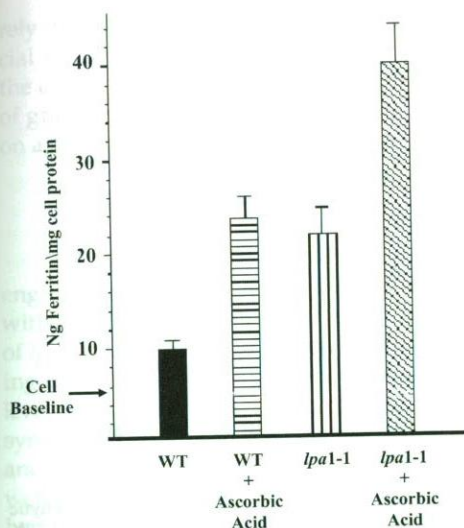


Fig. 8-4. Caco-2 Assay for iron bioavailability in grain produced by wild-type and *lpa1-1* maize isohybrids (Raboy, 2007; data provided by Ray Glahn, USDA-ARS Plant, Soil and Nutrition Lab, Cornell University, Ithaca, NY).

Iron absorption was 49% greater with *lpa* maize than wild-type (Mendoza et al., 1998). Another study was conducted to determine iron absorption in women fed porridge made from normal or *lpa1-1* maize which was fortified with ferrous sulfate or sodium iron EDTA. It was found that with the addition of the available form of iron added to the diets, after 12 d of consumption, there was no significant effect of phytate content on Fe absorption (Mendoza et al., 2001). As discussed above, these studies provide evidence that reducing the phytic acid of food crops can have the same benefit as adding iron supplement to the diet.

In small-scale clinical studies, low-phytate maize appeared to improve zinc absorption linearly as the phytic acid level consumed decreased. Six healthy adults were fed tortillas as their only food source for 2 d, prepared from maize isohybrids that were either Nutradense-LP (80% reduced phytic acid), *lpa1-1* (60% reduced phytic acid), or their respective "normal-phytate" isohybrids. Fractional absorption of zinc was 0.38 for Nutradense-LP, 0.28 for *lpa1-1*, and 0.15 and 0.13 for respective normal phytate isolines (Fig. 8-5) (Adams et al., 2002; Hambidge et al., 2004). However, in a larger-scale field study, only small, nonstatistically significant increases in Zn absorption were observed in human subjects consuming *lpa1-1* maize as compared with its normal-phytate control (Mazariegos et al., 2006). The central highlands of Guatemala were chosen as a study location because of the known low zinc status of its inhabitants as well as a diet where maize contributes 50% of the caloric intake. For 10 wk, three groups of 20 school-aged children were provided either *lpa1-1* maize, its normal-phytate isohybrid, or a local maize landrace. Fractional zinc absorption was 14 and 10% greater from the *lpa1-1* maize than from its isohybrid control or the local landrace, respectively, but this increase was not statistically significant at the $P = 0.05$ level. It was suggested that the results may have been affected by the fact that there were other possible sources of phytic acid and zinc in the foods consumed by the test subjects, including but not limited to zinc-fortified wheat products. As a result, while the reductions in grain phytic acid in the *lpa1-1* maize, as compared with the its normal-phytate isohybrid or the local landrace, were as expected, ranging from 65

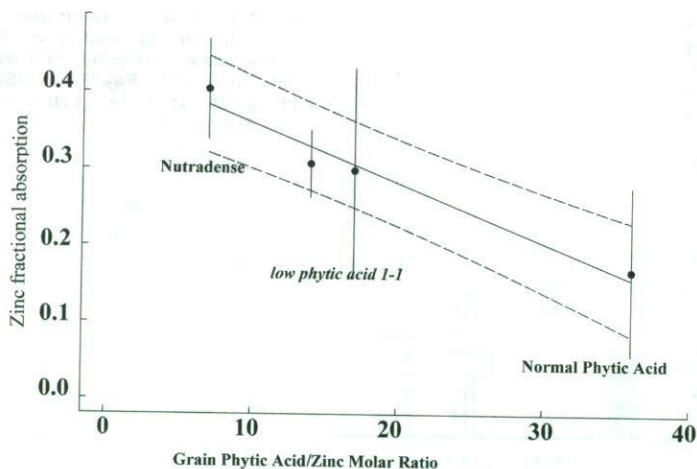


Fig. 8-5. Zinc fractional absorption in six healthy adults fed tortillas prepared from a normal phytic acid maize and two maize *low phytic acid* genotypes with phytic acid reductions of 60 and 80% (from Hambidge et al., 2004).

to 68%, the reduction in total phytate consumed daily by the test subjects provided with *lpa1-1* maize versus the other two normal-phytate types was only 25 to 32%.

In another short-term, small-scale clinical study with human subjects (Hambidge et al., 2005), calcium fractional absorption was enhanced in *lpa1-1* maize as compared with normal-phytate maize. For 2 d, five healthy adult women were fed breakfasts consisting of tortillas made with wild-type or *lpa1-1* maize. Although the Ca content was not different between the types of tortillas, Ca fractional absorption was 0.50 ± 0.03 for the *lpa1-1* tortilla as compared with 0.35 ± 0.07 for the wild-type grain tortilla. The increase in calcium absorption could be of practical importance when dairy intakes are limited or perhaps when cereals and legumes represent major components of diets.

While phytic acid is considered an antinutrient because of its role in reducing nutrient absorption, there is also evidence that phytic acid can act as an inhibitor of renal stone formation (Grases et al., 2000) or as an anticancer effects both in vitro and in vivo (Shamsuddin, 2002). Findings of the effect of phytic acid treatment on cancer cells include reduced frequency of colon tumor development in rats (Shamsuddin, 2002), reduced head and neck squamous cell cancer progression (Janus et al., 2007), and reduction of proliferation of pancreatic cancer in vitro (McMillan et al., 2007). Where do the contrasting antinutrient or prohealth effects of phytic acid leave the *lpa* crops? Consideration should be given to how much phytic acid is actually in the diet to determine if reducing it may help nutrient absorption. There is significant variation in phytic acid consumption around the world. For example, in China average daily phytic acid consumption is 1433 mg in rural areas and 648 mg in large cities (Ma et al., 2007).

In the USA, the average consumption is 750 mg per day, 219 mg per day in Italy (Carnovale et al., 1987), up to 2200 mg per day in Nigeria (Harland et al., 1988), and 3200 mg per day in Guatemala (Hambidge, personal communication). Also, the health-negative effects relate more to infant-child growth and to women of child-bearing age and to communities in the developing world that

rely more often on diets rich in cereals and legumes, whereas the health-beneficial roles relate more to health concerns in aging and in diets more common in the developed world. Therefore the health-beneficial versus antinutritional roles of grain- and legume-derived dietary phytic acid probably should be evaluated on a case-by-case basis.

Summary and Future Directions

As a result of the use of both classical genetics–breeding approaches and bioengineering approaches, a growing tool box of resources are now available to deal with problems associated with seed phytic acid. This includes a growing number of *lpa* loci and alleles in a growing number of crop species as well as an increasing number of molecular genetic tools useful for engineering seed phytic acid levels, such as phytase constructs designed to be targeted to the site of phytic acid synthesis. The benefit of a low-phytate feed in nonruminant livestock production, and the need to deal with phytate-related issues in livestock production, have been well documented. More work is needed in the area of human nutrition. This includes better documentation of dietary phytate's putative positive roles and the clarification of where manipulation of dietary phytic acid might or might not be desirable.

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